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Effect of pre-exposure to methamphetamine in male rats at two training levels
subthreshold to habit

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April 2020

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Abstract

Operant conditioning is a type of learning where behaviors become more or less likely to reoccur after being rewarded or punished. Habits are motor responses performed automatically in response to a particular stimulus, which can be adaptive because it frees up cognitive workspace for other tasks. Habits form after repeated pairings of the behavior with a desirable outcome, and eventually the behavior will occur even when the outcome is no longer rewarding. The parallel to addiction is not coincidental, as substance abuse is thought to change the networks involved with habit. However, the neural circuitry underlying the transition in behavior from goal-directed to environment-elicited is not completely understood. Exposure to psychostimulants prior to training has been shown to decrease the number of reward exposures needed during operant training for an animal to begin to respond habitually rather than in a goal-directed manner. This study investigated the effect of pre-exposure to methamphetamine on Long Evans male rats with 120 and 160 response-reinforcer exposures on a variable-interval 30-s schedule, which is a level of reinforcement subthreshold to habit in male rats. Following pairing of the sugar-pellet reward with taste-aversive lithium chloride, the rate of responding (nose-poke behavior) for both the methamphetamine pre-treated and control groups showed that both were sensitive to reward devaluation. This implies that the methamphetamine pre-treatment did not accelerate habit formation at either level of training. While this is the same result as a previous study with female rats, it does indicate a divergence from the literature which indicates that pre-exposure with psychostimulants accelerates habit formation in males.

Introduction

We do things subconsciously every day: roll over and shut off the alarm in the morning, walk into a room and turn on the light even if it's not needed, drive the same way to work and accidentally do it when you meant to run errands. These are habits; the automatic behaviors formed through repetition of a behavior in a particular context. A lot of the time, habits are beneficial because they free up cognitive capacity for other tasks by delegating routine behaviors to the subconscious. However, when this system is hijacked by maladaptive behaviors, anything from teeth grinding to heroin use, habits can become harmful. Of course, we're not robots that can't change our behavior just because we repeat it a few times, but this learning process is complex and can be influenced by many factors.

Instrumental learning, or operant conditioning, is the process of reinforcing or punishing a certain behavior and making it either more or less likely to reoccur. This is a very common and basic form of learning, and if it is reinforced enough, eventually the behavior will occur without a reward. In the 1980's, a number of important experiments found that overtraining rats in an operant paradigm resulted in decreased sensitivity to reductions in the value of the reward (Dickinson, 1985). A habit is often operationally defined as insensitivity to reward devaluation and falls in contrast to an action, a goal-directed behavior that is performed because it has become associated with a rewarding outcome. When the value of the reward is reduced, the action frequency decreases because the positive association between the action and the reward is disrupted. With enough repetitions, a behavior that was goal-directed becomes habitual. During an instrumental learning task, there are thought to be two parallel systems at play: action-outcome (A-O) learning, whereby when the behavior (action) is performed to achieve a goal (outcome), and stimulus-response (S-R) learning, in which the learned behavior (response)

becomes associated with contextual stimuli (like the environment). The contrast between goal-directed and habitual behaviors, as tested by devaluation of the reward, is thought to depend on which of these systems has more influence at the time of testing. S-R learning becomes stronger with more repetitions and eventually, no motivational drive is needed and the environmental cues are enough to elicit the motor behavior (Dickinson et al., 1995; Everitt & Robbins, 2005).

Unsurprisingly, different areas of the brain are involved in different kinds of learning. Numerous lesion studies have shown that the dorsolateral striatum (DLS), central nucleus of the amygdala, infralimbic cortex, and the substantia nigra are important in forming and performing habits (Corbit et al., 2013; Smith & Graybiel, 2016; Yin et al., 2004). In contrast, A-O learning is mediated by the dorsomedial striatum (DMS), prelimbic cortex, and orbitofrontal cortex (Corbit et al., 2013; Smith & Graybiel, 2016). Generally, the striatum is the key region where the various cortical inputs influencing motor patterns are sorted and fed back to the cortex through the thalamus (Haber, 2016).

On a cellular level, groups of neurons in the DLS and infralimbic cortex exhibit increased activity at certain points throughout the action, notably at the beginning and end and not in the middle. This has been termed “chunking”, a pattern which diminishes when the reward is devalued, but is stable in other changes to the task (Graybiel & Grafton, 2015; Smith & Graybiel, 2016). There is also evidence that as the behavior shifts from goal-directed to habitual, fewer neurons are required to initiate and carry out the behavior (Tang et al., 2007) until ultimately multiple motor programs are initiated together by only a few neurons. However, this chunking behavior is not seen in the DMS or other A-O regions, and the DLS shows other responses to the outcome, separate from chunking, so it is not clear what exact functions all these regions play in habit (Smith & Graybiel, 2016). While obviously there are many interneurons and collaterals that

connect the various regions, the DLS is part of the sensorimotor corticostriatal loop which receives glutamatergic input from the sensory and motor cortices and dopamine from the substantia nigra (SNc). In contrast, the DMS gets more input from the prefrontal cortex and dopamine from the ventral tegmental area and less from the SNc. Though they work in parallel, primary control over a behavior seems to move from an action to a habit with a shift from the DMS to the DLS (reviewed in Canales, 2005). Most research uses rodent or non-human primate models to study this process, but similar brain regions are shown to be involved in humans too. For example, cue sensitivity with increased training is reflected in more activity in the dorsal putamen, the human equivalent of the rodent DLS (Tricomi et al., 2009).

Medium spiny (projection) neurons (MSN's) are the main integrative unit of the striatum and many drugs of abuse directly act on dopaminergic inputs that modulate the firing of these neurons. As reviewed in Gremel and Lovinger (2017), addictive drugs both acutely and chronically change the neuronal structure and functioning of areas including the nucleus accumbens, substantia nigra, and the dorsal striatum. In particular, psychostimulants like amphetamine, methamphetamine, and cocaine increase dopamine in the synapse by blocking or reversing the dopamine transporter which results in changes in motor behavior through actions in the striatum. Few studies distinguish between changes in the DMS and the DLS, but Jedynak et al. (2007) found that after a month-long exposure to methamphetamine spine density increased in the DLS but decreased in the DMS which may reflect changes in corticostriatal control through AMPA receptors and growth of new NMDA-containing spines. A more recent study found that a nearly-identical regime of methamphetamine treatment increased NMDA and the GluR1 subunit of AMPA receptors in the DLS and decreased glutamate-related proteins in the DMS (Furlong et

al., 2018). These changes in glutamate activity likely reflect synaptic plasticity and perhaps the changing influence of various inputs on MSN's.

In conjunction with these neurobiological studies, several investigators have also examined the effects of psychostimulants on the behaviors mediated by these brain regions, including actions and habits. Among these studies is one by Nelson and Killcross (2006) who gave minimal operant training to male rats (a level subthreshold to habit) and found that those that were pre-treated with a low dose of D-amphetamine showed insensitivity to devaluation (i.e. were habitual) while the control group remained sensitive (goal-directed). In a follow-up experiment in the same study, rats were sensitized post-training, and it was found that all groups remained goal-directed, implying that the amphetamine pre-treatment facilitated habit acquisition. A study by Furlong et al. (2018) found similar results using methamphetamine: drug pre-treatment promoted habit formation, although the length and dosing of exposure was much higher. Other studies in male rats with cocaine have found that it facilitates habit formation when given before training (Corbit et al., 2014; LeBlanc et al., 2013), after training (Schmitzer-Torbert et al., 2015), and that it too changes glutamate homeostasis, though in the DMS rather than the DLS (Corbit et al., 2014).

The purpose of the present study was to examine the effect of methamphetamine pre-exposure on the formation of habitual behavior using our laboratory's behavioral paradigm. This was tested with male rats trained to a level subthreshold to habit where the literature would predict that the rats would show an accelerated development of habitual behavior due to the drug pre-exposure. The tested levels of training were 120 and 160 response-reinforcer exposures, and both used devaluation with a taste-aversive agent (lithium chloride) to test for flexibility in the learned behavior (nose-poking). The levels of training were chosen to attempt to repeat the

finding of Nelson and Killcross (2006) (120 reinforcers) with a new drug, and because of an interesting effect in pre-treated females at 160 reinforcers, a point where they are normally habitual (unpublished results). For these experiments with males, it was expected that at least the 160 group, if not both, would show habitual nose-poking because of the pre-exposure to methamphetamine.

Methods

Animal Maintenance

Adult male Long Evans rats were obtained from Charles River Laboratories, Quebec, Canada. All animal care and experimental procedures were approved by the University of Vermont's Institutional Animal Care and Use Committee. Thirty-two animals were used in Experiment 1, while thirty-six were used in Experiment 2. In both experiments, animals were pair-housed in a climate-controlled colony room on a 12-hour light-dark cycle. The animals were acclimated to human contact and then placed on a food restricted diet at 85% of their *ad libitum* weight which was maintained throughout the entirety of the experiment. Animals were fed in the early afternoon while all other experimentation took place in the morning.

Methamphetamine Protocol

After acclimation, animals in both experiments were exposed to a sensitizing dose of methamphetamine (METH). Half of each cohort received intraperitoneal injections of 2.5 mg/kg methamphetamine hydrochloride (Sigma Aldrich). The other half received injections of 0.9% saline (vehicle group) at a volume equivalent to the average of the METH injections. Injections were carried out at the same time each day before feeding, at roughly the same time that operant training would later take place. The treatment was eight days in length, followed by three washout days to allow for any drug traces to leave their system.

Next, a sensitization test was conducted: all animals were administered a 0.3 mg/kg dose of methamphetamine. For Experiment 1, eight cages were selected, four that had received pre-exposure to METH, and four that received saline. Each cage was videotaped for one minute, 5 minutes after the injection, and a motor-behavioral analysis was carried out blind to the cage's METH condition. In Experiment 2, all of the animals were filmed and scored. Several traits are

typically associated with METH exposure (Becker & Hu, 2008) which have been corroborated by previous repetitions of this protocol. These include a “shimmying” movement where the rat stands on its hind legs and sways its shoulders; rapid, jerky movements; increased running back and forth across the cage; and repetitive head bobbing. Each instance of a stereotypical behavior was scored with a point, providing a total behavior count for animals in each drug pre-treatment condition. In both experiments, the average number of behavior counts was compared between treatment conditions to ensure that the group pretreated with METH displayed motor changes due to the psychostimulant, taken as an indication of changes to the striatal environment.

Instrumental Training

The operant training phase began the day following the sensitization test. The six operant chambers (Med Associates, St. Albans VT and accompanying MED-PC software) were housed within light-and-sound attenuating boxes which also contained the sugar-delivery mechanism. The animals were trained in the same box for each stage of the project to ensure a consistent learning context for the duration of the experiment. One wall of the chamber contained a hole where the animal could perform a nose-poke, and an adjacent, larger dish compartment (magazine port) where sugar pellets could be delivered. The magazine was present in all stages, while the nose-poke was removed as needed. A house light in the chamber was illuminated while the behavioral program was running, and shut off when the program completed.

Training began with two sessions of magazine training where the rats were placed in their assigned chamber for thirty minutes. Sucrose pellets, which were the reinforcers used for the duration of the experiment, were delivered on a variable time (VT) 60-second schedule, with the nose-poke devices physically blocked. The purpose of this training phase was to acclimate the

rats to the learning context, and allow them to learn the sound of the pellet falling into the magazine, as well as the positive (reinforcing) value of the sucrose.

The second phase consisted of two sessions of continuous reinforcement of the nose-poking behavior with a sugar pellet. As such, the nose-poke mechanism was installed, and each performance of the behavior resulted in the delivery of one sugar pellet. Animals received a total of 25 pellets per session for two sessions.

The final phase of operant training employed variable-interval (VI) reinforcement of nose-poking behavior on a 30-second schedule. Sensitivity to devaluation of the reward is dependent, in part, by the number of response-reinforcer exposures, which here is the number of times the nose-poke behavior is paired with the sugar reward (Adams, 1982). In changing to a variable-interval reinforcement schedule, the previous 1:1 relationship between responding and reinforcement loosens, allowing contextual stimuli associated with nose-poking to become more predictive of the reward (Dickinson et al., 1995). On the VI 30-s schedule animals received 40 pellets and the rate of nose-poking was tracked over each session. In Experiment 1, animals received three daily VI-30 sessions for a total of 120 reinforcers earned in the acquisition period. This degree of training has been shown to support goal-directed behavior in male rats (Dickinson et al., 1995; Nelson & Killcross, 2006; Schoenberg et al., 2019). In Experiment 2, animals received four sessions, earning 160 total reinforcers over the course of acquisition.

Reward devaluation followed operant training in a carefully counter-balanced methodology. Half of the animals in each drug condition were assigned to a devaluation group: “paired” or “unpaired”. Throughout devaluation, pellets were delivered on a VT 30-s schedule, and immediately following their consumption, all animals were subject to the same kind of injection. However, only half the cohort was delivered sugar pellets at a time: the paired group

received sugar on odd days while the unpaired group received sugar on even days. All animals were placed in the chambers every day, and all received injections each day, but the difference was in whether the delivery of sugar was paired with the injection of taste-aversive lithium chloride (LiCl). The injections were carried out immediately after delivery of the last pellet whereby, on odd days, all animals received 10 ml/kg intraperitoneal injections of 0.15 M lithium chloride (LiCl), and even days, 0.9% physiological saline. As such, all animals became nauseous together or simply bloated together. The first day, 40 pellets were delivered and immediately following their consumption, the LiCl injections were carried out. On day three, again 40 were delivered, but on this and each subsequent odd-numbered day, some pellets were not consumed. These leftover sugar pellets were counted for each paired animal, and the following day unpaired animals would receive the average number of pellets consumed by their paired counterparts. Reward devaluation continued until all the paired animals ate zero of the presented pellets. To counterbalance, the last day was an even-numbered saline day. It was verified that all the delivered pellets were consumed by the unpaired animals on all days. The nose-poke hole was not present during reward devaluation.

Extinction Test

On the day following the last round of reward devaluation, habitual nose-poking was assessed with an extinction test. In this phase, animals were placed in their operant chambers with the nose-poke available, but no pellets were delivered. The rate of nose-poking per minute was recorded for 12 minutes. This tests sensitivity to devaluation under extinction conditions.

Consumption Test

The consumption test was conducted the day following the extinction test. All animals were delivered 20 pellets on a VT 30-s schedule and the nose poke apparatus was not available.

The purpose of this step was a manipulation check to ensure that the paired animals were fully devalued, and would therefore reject all the delivered pellets, while those that were unpaired still would eat all available pellets.

Reacquisition

The last test was reacquisition, where the nose-poke was available and sugar pellets could be delivered on a VI 30-s schedule for 30 minutes. Reacquisition of a suppressed or forgotten behavior can be measured with the rate of responding over time, here in nose-poking per minute. Paired rats should stop responding because they reestablish the relationship between nose-poking and sugar and incorporate the memory that sugar makes them nauseous. Conversely, rats in the unpaired group continue responding because the value of the reward was not manipulated. This step thus also tests successful devaluation.

Experiment 1 (120 reinforcers) Results

One animal was an outlier in the extinction test and was therefore removed from analysis ($z=2.19$; Field, 2007). This left 31 animals for further analysis in Experiment 1.

Sensitization test

The mean behavior counts in the METH and vehicle groups were compared with an independent samples t-test. The METH pre-treatment group showed significantly more stereotypical behaviors ($M=6.00$, $SD=1.63$) than vehicle group ($M=1.25$, $SD=.96$; $t(6)=-5.02$, $p=.002$). From this we can assume that the group pre-treated with METH were in fact sensitized before instrumental training began.

Acquisition

During acquisition, all four treatment groups (METH paired and unpaired, vehicle paired and unpaired) acquired the nose-poking behavior. This was evaluated with an analysis of response rates in a 2 (drug pre-treatment group: METH or Vehicle) by 2 (anticipated devaluation group: paired or unpaired) by 3 (training session) repeated-measures ANOVA. Mauchly's test revealed a violation of sphericity ($\chi^2(2)=7.83$, $p=.020$), which was corrected using the Huynh-Feldt degrees of freedom ($\epsilon=.93$). There was a significant within-subjects effect of training session ($F(1.86, 50.16)=132.80$, $p<.001$), indicating that the level of nose-poking increased over the course of the three days (see Figure 1).

There was no significant difference in the between-subjects analysis of nose-poking rates by anticipated devaluation group ($F(1, 27)=.03$, $p=.863$), indicating the assigned paired and unpaired groups learned the behavior equally well. There was a marginally-significant, between-subjects effect of drug pretreatment group ($F(1, 27)=3.31$, $p=.080$), with the METH-pre-treated group trending towards higher rates of nose-poking (Figure 1).

Figure 1.

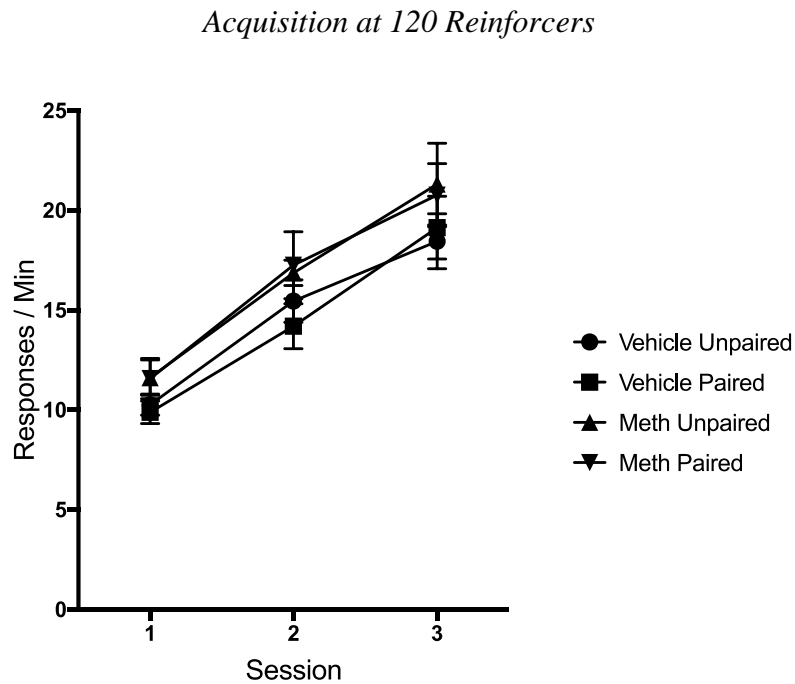


Figure 1 shows the increasing rate of nose-poking over the three sessions of instrumental training, indicating that the operant behavior was acquired.

Reward Devaluation

All animals in the paired group (received LiCl on the same days as were delivered sugar pellets) reached the criterion of zero pellets consumed by the 11th day. In contrast, all unpaired animals consumed all delivered pellets throughout this phase (see Figure 2).

Extinction

Response rates during the extinction test were analyzed in a 2 (drug pre-treatment group: METH or Vehicle) by 2 (devaluation group: paired or unpaired) factorial ANOVA. This analysis revealed a significant main effect of devaluation group ($F(1, 27)=30.75, p<.001$), with both paired groups responding significantly less than their unpaired counterparts. There was no effect of drug pre-treatment group ($F(1,27)=.003, p=.955$) nor a drug pre-treatment by devaluation group interaction ($F(1,27)=.10, p=.749$), indicating that both the vehicle and METH pre-treated

groups remained sensitive to devaluation and therefore goal-directed at 120 response-reinforcer exposures (see Figure 3).

Figure 2.

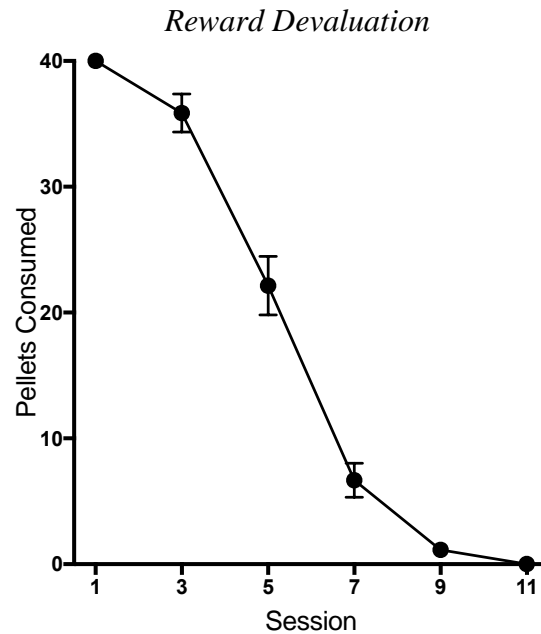


Figure 2 shows the drop in the number of pellets consumed by the paired animals over 6 sessions as the sugar was devalued with LiCl.

Figure 3.

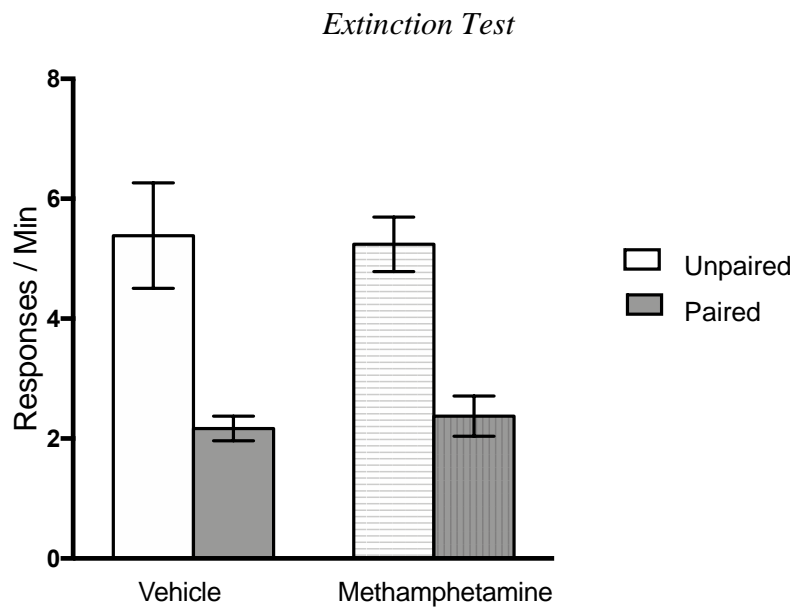


Figure 3 shows the results of the extinction test after training at 120 reinforcers. The decreased rate of responding in the paired animals indicates goal-directed behavior in both the vehicle and METH pre-treated groups.

Consumption

The paired animals consumed zero of the 20 delivered pellets while all unpaired animals ate all delivered pellets. This confirms that the paired group successfully acquired taste aversion to the pellets while the unpaired did not.

Reacquisition

This last test used the rate of nose-poking over 30 minutes, divided into averages of 5-minute bins. Results were analyzed with a 6 (time: 5-minute bins) by 2 (drug pretreatment: METH or Vehicle) by 2 (devaluation group: paired or unpaired) repeated measures ANOVA. Mauchly's test revealed a violation of sphericity ($\chi^2(14)=52.84, p<.001$), which was corrected using the Greenhouse-Geisser corrections for degrees of freedom ($\epsilon=.53$). There was a significant within-subjects effect of time ($F(2.63, 70.88)=38.05, p<.001$), indicating that the level of nose-poking was significantly different for each 5-minute bin (see Figure 4).

There was a significant between-subjects main effect of pairing ($F(1, 27)=122.29, p<.001$), with the paired groups responding less than unpaired groups. There was also a significant interaction effect of devaluation condition with time bin ($F(2.63, 70.88)=68.85, p<.001$), with the difference between the paired and unpaired groups becoming larger over the 30 minutes (see Figure 4).

Figure 4.

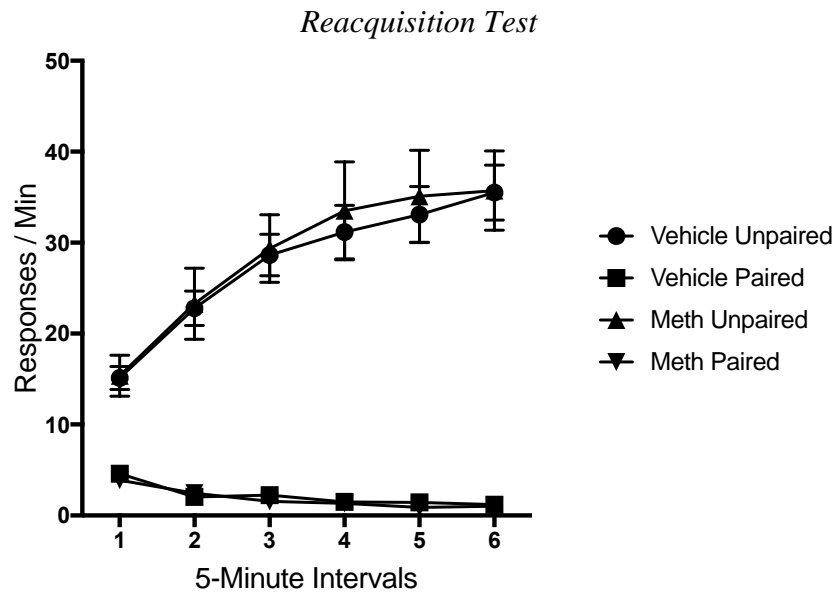


Figure 4 shows the increasing split in nose-poking rates over the 30-minute reacquisition session with the unpaired animals responding more. There was no significant effect of drug treatment compared to controls of the same devaluation group.

Experiment 2 (160 reinforcers) Results

One animal was an outlier in the extinction test and was removed from analysis ($z=2.23$; Field, 2007). This left 35 animals for further analysis in Experiment 2.

Acquisition

The nose-poking behavior during instrumental training was analyzed using a 4 (training session) by 2 (drug pre-treatment: METH or Vehicle) by 2 (anticipated devaluation group: paired or unpaired) repeated measures ANOVA. Mauchly's test revealed a violation of sphericity ($\chi^2(5)=22.00, p=.001$), therefore Huynh-Feldt corrections for degrees of freedom were employed ($\epsilon=.83$). There was a significant within-subjects effect of training session ($F(2.48, 76.81)=161.01, p<.001$), indicating that the rate of the instrumental behavior increased over the four sessions (see Figure 5).

There was no significant difference in the between-subjects analysis of nose-poking rates by anticipated devaluation group ($F(1, 31)=2.04, p=.163$), nor drug pre-treatment group ($F(1, 31)=.33, p=.567$). From this we can assume that all groups acquired nose-poking equivalently (see Figure 5).

Figure 5.

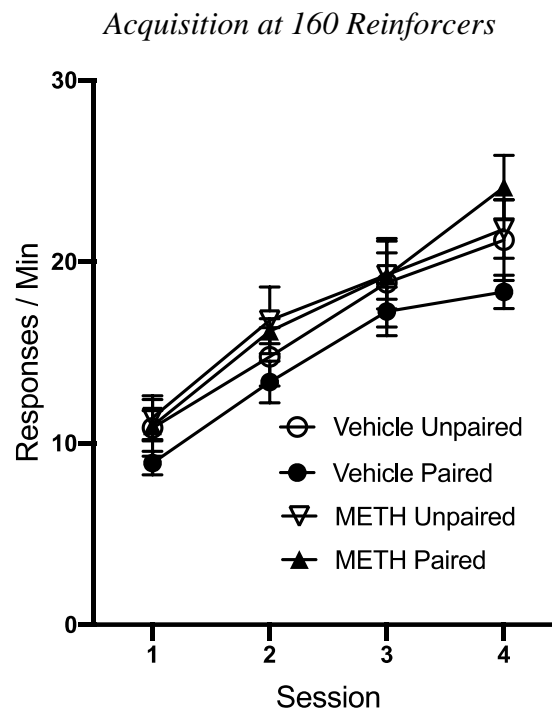


Figure 5 shows the increasing rate of nose-poking over the four sessions of instrumental training, indicating learning of the operant behavior.

Reward Devaluation

All animals in the paired group reached the criterion of zero pellets after 11 days of devaluation. All unpaired animals consumed all delivered pellets throughout this phase (see Figure 6).

Figure 6.

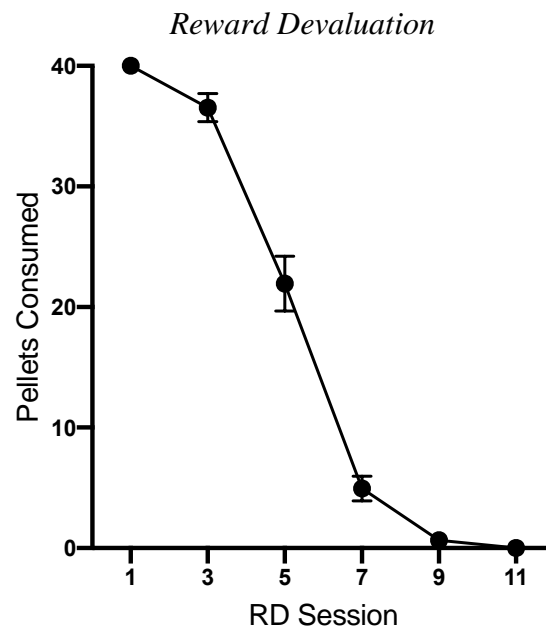


Figure 6 shows the decreasing number of pellets consumed by the paired animals over the 11 sessions of devaluation with LiCl.

Extinction

The rates of nose poking during extinction were analyzed in a 2 (drug pre-treatment group: METH or Vehicle) by 2 (devaluation group: paired or unpaired) factorial ANOVA. Results from this analysis showed a significant main effect of devaluation group ($F(1, 31)=14.34, p=.001$). There was no effect of drug pre-treatment ($F(1,31)=1.52, p=.228$), nor drug pre-treatment by devaluation group interaction ($F(1,31)=.29, p=.592$). So, as both paired groups responded significantly less than the unpaired animals, both the vehicle and METH pretreated groups remained sensitive to devaluation and therefore goal-directed at 160 response-reinforcer exposures (see Figure 7).

Figure 7.

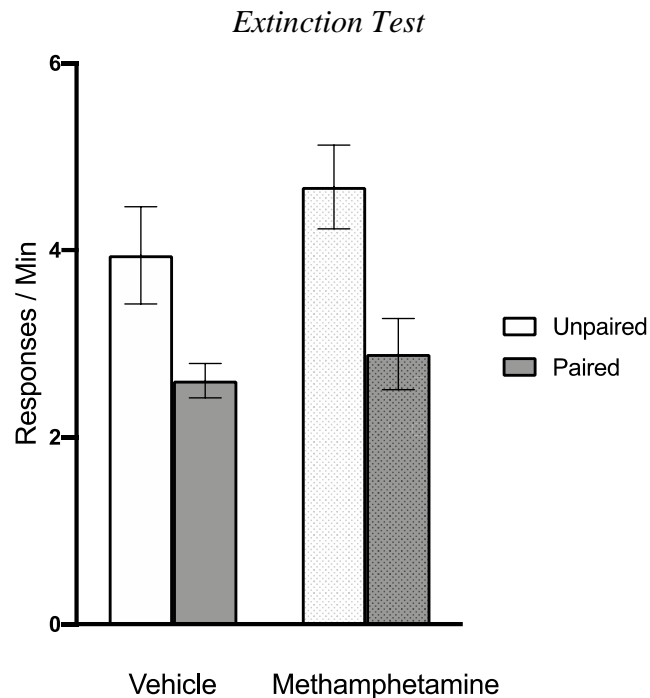


Figure 7 shows the lower rates of nose-poking in both the paired groups, and there was no effect of drug pre-treatment.

Consumption

All paired animals consumed zero of the 20 delivered pellets while all unpaired animals ate all delivered pellets. This confirms that animals in the paired group were successfully conditioned to the taste aversion of the “sugar pellets” with LiCl.

Reacquisition

The rates of nose-poking over 30 minutes were analyzed with a 6 (5-minute bin) by 2 (drug pre-treatment: METH or Vehicle) by 2 (devaluation group: paired or unpaired) repeated measures ANOVA. Mauchly's test revealed a violation of sphericity ($\chi^2(14)=61.18, p<.001$), which was corrected using the Greenhouse-Geisser corrections for degrees of freedom ($\epsilon=.51$). There was a significant within-subjects effect of reacquisition bin ($F(2.53, 78.47)=38.92, p<.001$), indicating that the level of nose-poking was significantly different for each 5-minute bin (see Figure 8).

There was significant between-subject effect of pairing ($F(1, 31)=135.05, p<.001$), with the paired groups responding less than unpaired groups, which confirmed successful devaluation. There was also a significant interaction effect of time with devaluation condition ($F(2.53, 78.47)=67.09, p<.001$), with the gap between the paired and unpaired groups growing larger over the 30 minutes (see Figure 8).

Figure 8.

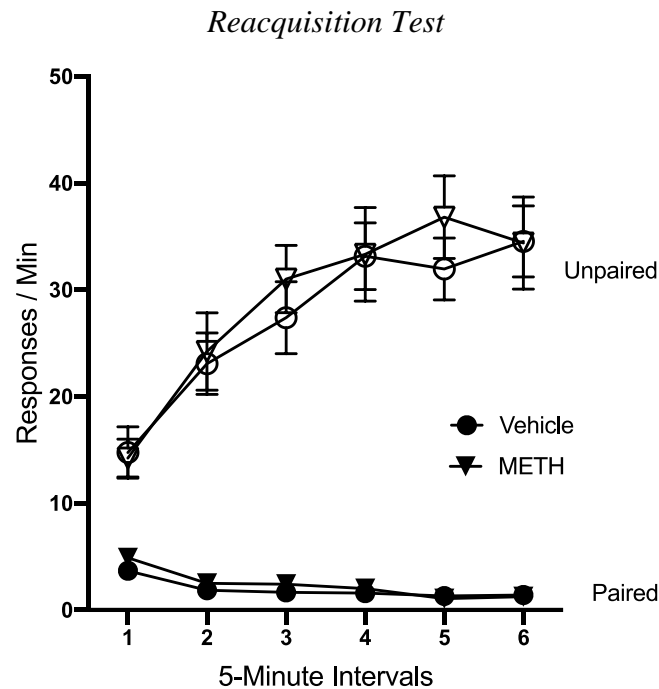


Figure 8 depicts the reacquisition test for Experiment 2 where animals in the unpaired group increased responding over the session and paired animals decreased. There was no effect of drug pre-treatment.

Discussion

The purpose of the present study was to investigate the formation of habitual behavior after pre-exposure with methamphetamine in male rats. Despite successful sensitization to METH, acquisition of the operant behavior, and devaluation, the extinction tests showed significantly lower responding in both the vehicle and METH-sensitized animals compared to the unpaired groups, indicating that goal-directed behavior was maintained. This lead us to conclude that exposure to METH prior to training failed to advance habitual responding at both 120 and 160 response-reinforcer exposures.

There are several possible reasons for this finding. First, though our methodology is modelled after the literature, it is still difficult to compare across studies. The primary study that influenced the choice of psychostimulants to change striatal function was Nelson and Killcross (2006). They employed D-amphetamine at a non-addictive dose for one week, both before and after instrumental training in separate experiments. The post-training drug treatment did not change response rates during extinction but the pre-training treatment group showed habitual responding. This showed that the drug exposure had no impact on learning ability, as we also demonstrated, but that it needed to come before training to have an effect. However, their amphetamine pre-treatment induced insensitivity to devaluation while it did not in our study at the same level of training (120 reinforcers). This is interesting in light of experiments both *in vivo* and *in vitro* showing that METH releases up to five times more dopamine into the synapse than amphetamine, with both acting on the dopamine transporter (Goodwin et al., 2009). We would expect that, because METH is a stronger psychostimulant, a similar dose would result in the same, if not more pronounced behavioral changes. Methodological differences may account for the disparity in findings, namely that taste-aversive devaluation lasted only 3 days, the study

lacked a consumption test, and there was no counterbalancing for nausea. In general, the protocol employed by our experiment has a more thorough devaluation procedure to ensure that all paired animals are fully conditioned to the taste aversive “sugar” so that any responding in extinction conditions is not due to remaining goal-directed motivation towards the reward. Furthermore, counterbalancing the LiCl and saline days is important because nausea is stressful, so stressing only the paired animals may be confounding because stress has been shown to push animals towards habitual responding (Packard, 2009) and increase spine density in the DLS, also associated with habit (Taylor et al., 2014).

Other studies of habit with psychostimulants have similarly found accelerated habit formation after pre-treatment with drugs of abuse while untreated animals remain goal-directed. With a slightly lower dose of amphetamine and satiety-specific devaluation, Nordquist et al. (2007) found accelerated habit learning, as did Corbit et al. (2014) with cocaine. Furlong et al. (2018) is one of the few studies that have used METH, and while they found an acceleration of habit, they used a much longer and higher dose than our study, and again there were only three days of devaluation, no counterbalancing of LiCl, and no consumption test. Furthermore, many studies give the drug after or concurrent with training (not as the reward) (LeBlanc et al., 2013; Schmitzer-Torbert et al., 2015) or have training paradigms that are not easily comparable to ours because of multiple instrumental trainings, step-wise interval training, or designs that intentionally discourage habit formation in pre-treated animals (Halbout et al., 2016). In sum, while other studies may find that psychostimulants change the striatum in a way that appears to be habit-promoting, less thorough and balanced devaluation measures may mean their results over-state the actual degree of behavioral change.

The second potential reason for not finding habitual behavior at either level of training may be simply that it wasn't nearly enough with our paradigm. Adams (1982) found that manipulating the number of response-reinforcer exposures can reveal goal-directed or habitual behaviors, and Dickinson et al. (1995) found that at 120 reinforcers the male rats were goal-directed while at 360 they were habitual. With our laboratory's more extensive process of devaluation, training males to 240 reinforcers was insufficient for habit (Schoenberg et al., 2019). Thus, the levels of training chosen for this study were intentionally subthreshold to habit in males to match our prior research and the literature, but it may have been too low to see any effect. The obvious next step would be to find the "tipping point" in males without the influence of METH, then to repeat this process at a level of training closer to the goal-to-habit transition to see if pre-treatment with METH advances habit when within a more appropriate range.

The third explanation for this result is that pre-exposure to METH may not actually push animals towards habit. This would be totally in contrast to the psychostimulant literature on habit, but unpublished results from our lab have found that female rats, who are typically habitual when trained to 160 response-reinforcer exposures, appear to remain goal-directed when pre-treated with METH. If this is also the case for males, a better experiment would be, after finding the range where the goal-to-habit transition occurs, to test at its higher end to see if METH also delays habit in males. While METH changes the physiology of the striatum, the relative strength of the dose may impact synaptic plasticity in a way that slows the shift in control from the DMS to the DLS, boosts control in the DMS, inhibits the DLS, or some combination of these options. Rats trained after pre-treatment with an intentionally neurotoxic dose of METH showed impaired S-R but normal A-O learning and depleted dopamine in both the DMS and DLS (Son et al., 2011). While our dose was not neurotoxic, the thresholds for

dopamine-enhancing or cytotoxicity are not well known, and a behavioral assessment can only reveal so much about the biological changes METH induces.

To be clear, the present study did not study addiction, but rather habit after manipulation with a drug of abuse that changes dopamine activity in the DLS. Studies that use substances of abuse as the reward during instrumental training, rather than sugar or some other food reinforcer, clearly are better simulations of drug use in an animal model. While it has been found that cocaine (Vandaele et al., 2019) and alcohol (O'Tousa & Grahame, 2014) speed habit formation when given as the reward, the behavior is almost immediately insensitive to changes in the reward, making it hard to devalue the drug and evaluate habit. Some studies of self-administered drugs were successful in devaluing the drug on a limited time frame, while extended experience was insensitive to devaluation unless agonists or lesions of the relevant striatal systems were involved (reviewed in Smith & Laiks, 2018). Nevertheless, habit research is applicable to addiction in addition to other pathologies.

This experiment demonstrated that the METH treatment successfully sensitized male rats. However, drug treatment prior to instrumental learning at both 120 and 160 response-reinforcer exposures was not enough to accelerate habit. Both vehicle and METH animals remained goal-directed despite evidence from amphetamine, cocaine, and methamphetamine studies indicating that animals show increased habitual responding at levels of training sub-threshold to habit without the drug. Though variations in methodology make comparing these studies difficult, possible reasons for our findings include differences in dosing of the psychostimulants, training procedures, devaluation procedures, stress between animal groups, insufficient levels of training, and the possibility that methamphetamine does not advance habit at all.

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